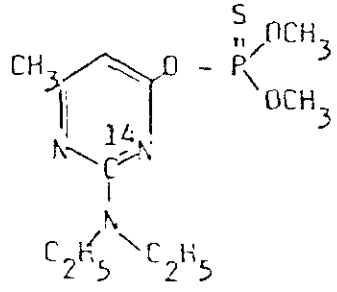
PIRIMIPHOS-METHYL - METABOLISM IN HENS.

In an earlier study ^{14}C-pirimiphos-methyl (0-2-diethylamino-6-methylpyrimidin-4-yl/0,0-dimethyl phosphorothiote) was administered orally to laying hens at rates equivalent to dietary concentrations of 4 mg/kg and 32 mg/kg (1). At the higher dose level radioactive residues of up to 0.4 mg/kg and 0.15 mg/kg were detected in the meat and eggs from the treated hens. However, in this study these radioactive residues were not characterised. Additional work has now been carried out to identify these residues and the results obtained are summarised below.

Treatment of Hens.

Two hens were dosed orally for 14 consecutive days with ^{14}C-pirimiphos- methyl at a rate equivalent to 7 mg/kg in their diet.



Position of radiolabel in ^{14}C-pirimiphos-methyl.

Eggs were collected from the hens throughout the dosing period. Four hours after the final dose the hens were sacrificed and samples of meat taken.

Results

a. Residues in meat

At sacrifice the radioactive residues in the meat of the two hens was 0.39 mg/kg. Most of this residue ($\sim$ 90%) was extractable with water and after "clean-up" a fraction of this extract (equivalent to 76% of the residue in the meat) was analysed by thin layer chromatography (TLC). The results obtained (see fig.6) showed that the residue consisted mainly of polar material chromatographing at the origin ($\sim$ 50%), compound III* ($\sim$ 9%) and compound IV plus an unknown which chromatographs just ahead of compound IV (together representing 27%). Traces of compound II were also detected.

* Structures of all compounds are given in figure 1.

After hydrolysis with 2M hydrochloric acid, a fraction equivalent to 65% of the total residue in the meat was analysed by TLC. (see fig. 7). No significant change in the relative amounts of Compounds II, II and V, was detected in the extract. However, the polar material, and the unknown chromtographing just ahead of Compound IV were no longer present, but the percentage of Compound IV in the fraction analysed had increased to 73%.

These results, therefore, indicate that the residue in the meat is mainly due to free and conjugated Compound IV, with small amounts of Compound III and traces of Compounds II and V.

Mass spectrographic analysis confirmed the identification of Compound IV (free and conjugated) as the major component of the residue in the meat, and reverse isotope dilution analysis showed that 78% of the extractable radioactivity was due to free or conjugated Compound IV.

b. Residues in the Eggs

The radioactive residues in the eggs from the treated hens ranged from 0.02 - 0.06 mg/kg in the albumen and from 0.01 - 0.08 mg/kg in the yolk.

Chromatographic analysis of a methanol extract of the albumen (equivalent to 82% of the total residue) indicated that this residue consisted of at least 7 different compounds (see Fig.11). Accurate quantification of the relative amounts of the radioactive compounds present in the extract was difficult because of the small amounts that were present. However, zonal scraping of chromatograms and HPLC analysis indicated that Compound III(12-24%) and Compound IV (13-15%) were the major identifiable compounds present, and a trace of Compound II was also detected. No discrete unidentified radioactive compound was detected on the chromatograms which represented $>$ 20% of the total extract.

Hydrolysis of the albumen extract with 2M-hydrochloric acid did not appear to cause any major changes in the relative amounts of the radioactive compounds present (See fig. 12). Although a small amount of Compound V (8%) was detected in the hydrolysed extract, which was not present prior to hydrolysis.

Extraction and chromatographic analysis of the yolk was more difficult than for the albumen because of interference from co-extractives. However, chromatographic analysis (see fig.14) of a methanol extract(equivalent to 54% of the total residue in the yolk) showed that Compound III (33%) and Compound IV (15-16%) were the major identifiable compounds present, with a small amount of Compound II (4 - 5%) also being detected. As with the albumen, the remainder of the radioactive residue consisted of a number of unidentified compounds (at least 5), none of which represented $>$ 15% of the radioactivity in the extract.

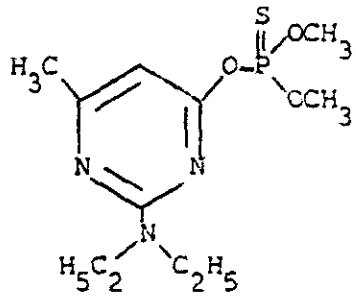
Two other fractions (a hexane extract, and an acid extract obtained by refluxing, with 5M-hydrochloric acid, the solid residue remaining after solvent extraction) each representing ~ 10% of the residue in the yolk, were also analysed. Pirimiphos-methyl was characterised as the major radioactive compound (60%) in the hexane extract and Compound II (12%), Compound III (22%), Compound IV (4%) and Compound V (6%) were detected in the acid extract.

The radioactive residue in the eggs is, therefore, due to a fairly complex mixture of compounds, with compounds III and IV as the major identifiable components.

REFERENCES

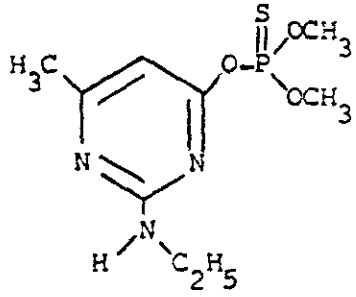
1. Green T, Monks I H, Phillips P J, Toxicological Report HO/IH/P/65B (ref. 19D Petition for Tolerance for the Admixture of 'Actellic' 7E Insecticide on Farmer Stock Peanuts Accession No. 097674).

FIGURE 1 Structures of Reference Compounds



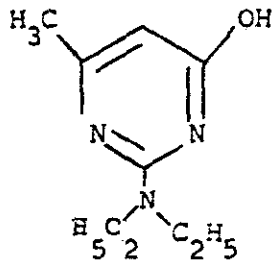
Pirimiphos-methyl:

O-2-diethylamino-6-methyl-pyrimidin-4-yl OO-dimethyl phosphorothioate



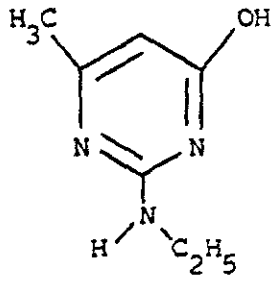
Compound I

O-2-ethylamino-6-methyl-pyrimidin-4-yl OO-dimethyl phosphorothioate



Compound II

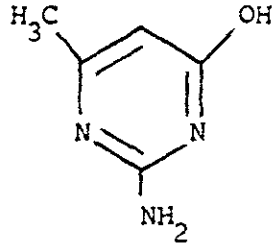
2-diethylamino-6-methyl-pyrimidin-4-ol



Compound III

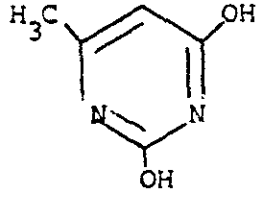
2-ethylamino-6-methyl-pyrimidin-4-ol

FIGURE 1 cont.



Compound IV

2-amino-6-methyl-pyrimidin-4-ol



Compound V

2,4-dihydroxy-6-methyl-pyrimidine